Localization of Nuclear Pore Complex in Hydrated Tumor Mammary Epithelial Cells by High Resolution Transmission X-ray Microscopy

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In order to evaluate the ability for soft x-ray microscopy to reveal well known nuclear structures, we have undertaken a series of immunolabeling experiments for proteins localized in defined nuclear subdomains. Here we present results obtained with staining for nuclear pore complexes, the visualization of which is particularly challenging in conventional electron microscopy. Nuclear pore complex (NPC) is a large (50-100x106 D) collection of proteins which organize the ~9 nm openings in nuclear membranes of eukaryotic cells. NPC immunolabeling was performed in tumor human mammary epithelial (T4) cells followed by imaging of whole hydrated labeled cells with a high resolution transmission x-ray microscope[1] (XM-1) (ALS Beamline 6.1.2). Cells

Figure 1 A. Human tumor mammary epithelial cell (T4) nucleus seen with XM-1. The nuclear pore complex (NPC) was immunolabeled with silver enhanced gold and appears uniformly distributed in a pattern characteristic of NPC.

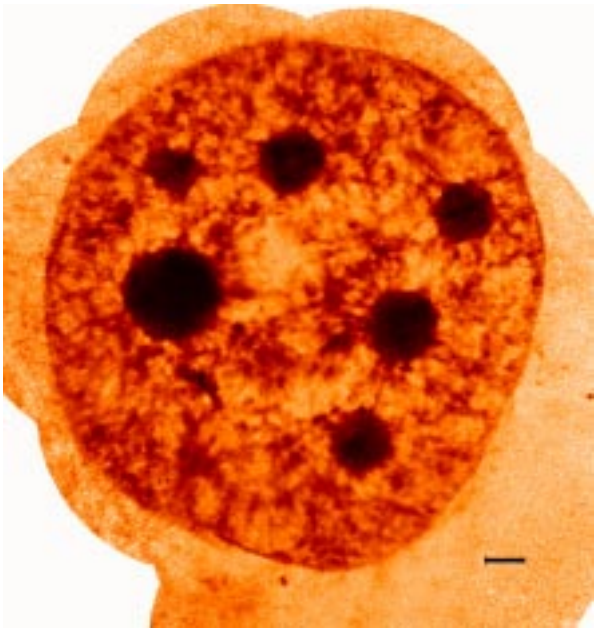

Figure 1 B: Control image of a cell prepared as for fig. 1 A, but without the primary antibody against NPC, therefore no staining is evident.

were grown on silicon nitrate windows (100 nm thickness), permeabilized with 0.5% triton in cytoskeletal buffer with protease and phosphatase inhibitors, and chemically fixed in 2% paraformaldehyde and 0.1% gluteraldehyde in phosphate buffered saline (PBS). The windows were then preincubated with superblock in PBS to block nonspecific labeling and incubated with anti-NPC polyclonal antibodies, followed by secondary labeling with a goat anti-rabbit antibody conjugated with fluorescein/nanogold (1:200 dilution). The fluorescein moiety allows imaging by confocal microscopy. Following detection of fluorescent labeling in the confocal microscope, the nanogold particles (1.4 nm diameter) were silver enhanced for viewing with the x-ray

microscope (spatial resolution 43 nm). XM-1 images of cells in an aqueous environment at atmospheric pressure were obtained using a 2.4 nm wavelength (517 eV photon energy level), below the K absorption edge for oxygen (543.1 eV) but above that for carbon (284.2 eV). Under these conditions, water is transparent while cellular structures and metallic labels are not. No staining was evident in control cells labeled with secondary antibody followed by silver enhancement (Fig 1 B). Silver enhanced label revealed numerous and uniformly distributed nuclear dots in cells labeled with both primary and secondary antibodies, a pattern that is characteristic of NPC (Fig 1 A).

REFERENCES

[1] W. Meyer-Ilse, E. Anderson, A. Nair, G. Denbeaux, L. E. Johnson, " Soft X-ray Microscopy Development at XM-1", (Berkeley Lab., Berkeley, 1999).

This work was supported by the Director, Office of Science, Office of Biological and Environmental Research and the Office of Basic Energy Sciences, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

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